

## 3-Isobutyl-2-methoxypyrazine is neutrally perceived by consumers at usual concentrations in French Sauvignon and Fer wines from the Gaillac area

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### ABSTRACT

3-isobutyl-2-methoxypyrazine (IBMP) is a grape-derived aroma compound responsible for the bell pepper character of wine. It is still unclear whether this molecule is always negatively perceived by consumers. The objective of this study was to establish a consumer rejection threshold (CRT) for IBMP in French white and red wines from the Gaillac area made from Sauvignon and Fer respectively.

The best estimate thresholds (BET) were determined by carrying out three-alternative forced choice (3-AFC) tests: 5.5 ng/L for Sauvignon and 16.8 ng/L for Fer. For the estimation of the CRT, consumers (n = 48) received pairs of samples consisting of a base wine and a base wine spiked with an ascending concentration of IBMP. They were asked to indicate which sample they preferred, and the CRT was calculated as the concentration for which the spiked sample was significantly rejected. CRTs were determined at 50 ng/L and 30 ng/L for Sauvignon and Fer respectively.

Our findings indicate that IBMP is more acceptable in white wine than in red wine. As IBMP concentrations reported in the literature for Sauvignon and Fer are generally below CRT concentrations, IBMP appears to be neutrally perceived by consumers at usual concentrations in wines from Gaillac made from these two cultivars. Our findings tend to contradict the belief in the wine industry that consumers systematically reject 'vegetative' styles of wines, and our results do not encourage local winegrowers and winemakers to necessarily implement viticultural or enological practices to minimise IBMP concentrations in wine.

### KEYWORDS

consumer rejection threshold (CRT), Sauvignon white wine, Fer red wine, 3-isobutyl-2-methoxypyrazine (IBMP), protected designation of origin Gaillac

## INTRODUCTION

The aromatic component of wine is an important element in consumer wine appreciation, as olfactory preferences are generally driven by the intensity and the dominant family of aroma perceived at tasting (Lesschaeve, 2008). In the 1980s, Noble *et al.* (1987) undertook to classify wine aroma into families leading to the development of a “wine aroma wheel”, which became a reference for wine writers, consumers, producers and researchers. From this work twelve distinct aroma families were identified, including a “vegetative” family related to sensory attributes, such as “grass cut green”, “eucalyptus”, “mint” and “bell pepper”.

The molecular determinism of these “vegetative” notes in wine has been widely studied and is currently well-understood. 3-Isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) are two potent grape-derived molecules contributing to the bell pepper and asparagus character respectively (Bayonove *et al.*, 1975). 3-alkyl-2-methoxypyrazines have been among the most studied aroma compounds since their first discovery in Cabernet Sauvignon (Bayonove *et al.*, 1975), and some years later in Sauvignon (Augustyn *et al.*, 1982). 1,8-cineole (also called eucalyptol) and its isomer 1,4-cineole, which impart eucalyptus and hay/dried herbs aromas respectively, have received a lot of attention from researchers in the past few years (Antalick *et al.*, 2015; Capone *et al.*, 2012; Poitou *et al.*, 2017). Although these monoterpenes can have an endogenous origin, it is also possible for them to be introduced to grapes and wines from neighbouring plants (Capone *et al.*, 2012; Poitou *et al.*, 2017). C<sub>6</sub>-compounds (i.e., *trans*-2-hexenal or hexanal), which are formed in the early winemaking stages through enzymatic oxidation of lipidic precursors, are responsible for the smell of cut grass in wines (Cordonnier and Bayonove, 1981). During alcoholic fermentation, a part of these compounds is reduced by yeast to an alcohol form or, for *trans*-2-hexenal, is involved in the synthesis of 3-mercaptohexanol (Roland *et al.*, 2010), which generally limits their sensory contribution to finished wines. Another monoterpene known as piperitone was recently identified as the main contributor of the mint nuances perceived in aged Bordeaux wines (Picard *et al.*, 2016; Pons *et al.*, 2016). It was also highlighted that *p*-menthane lactones can

enhance the freshness and the mint odours perceived in these wines (Picard *et al.*, 2017).

However, few research studies have been conducted to investigate consumer appreciation of the molecules responsible for these “vegetative” characters. The most significant study demonstrated that 1,8-cineole became undesirable in red wines above a concentration of 27.5 µg/L, thus encouraging winemakers to produce wines with moderate levels of this compound (Saliba *et al.*, 2009). Without performing a formal consumer study, piperitone was considered a positive aroma compound, as the molecule was positively correlated with aging-bouquet typicality scores (Picard *et al.*, 2016). 3-alkyl-2-methoxypyrazines, and notably IBMP, are usually seen as highly undesirable compounds by wine professionals and researchers. However, while the appreciation of methoxypyrazines has not yet been studied in red wines, moderate levels of IBMP can have a positive contribution to the aroma of Sauvignon wines (King *et al.*, 2011; Rajchl *et al.*, 2009).

The aim of this study was to formally assess the appreciation of IBMP in white and red wines by applying the consumer rejection threshold methodology (CRT) (Prescott *et al.*, 2005). This valuable approach, based on the application of the paired preference test, allows the concentration of a molecule that gives a significantly lower preference to be determined. It has been previously applied to several compounds or flavours, including those that cause wine faults, such as 2,4,6-trichloroanisole (Prescott *et al.*, 2005), 1,1,6-trimethyl-dehydronaphthalene also known as TDN (Ross *et al.*, 2014), ethyl phenyl-acetate and phenyl acetic acid (Campo *et al.*, 2012), 1,8-cineole (Saliba *et al.*, 2009) and rotundone (Geffroy *et al.*, 2018).

Sauvignon and Fer are two grape cultivars grown in the southwest of France within the Protected Designation of Origin (PDO) Gaillac, which cover 500 and 1000 ha respectively, according to the local winegrower association ([www.vins-gaillac.com](http://www.vins-gaillac.com)). Wines made from these two grape cultivars, which are known to be regular IBMP producers (Allen *et al.*, 1991; Davaux, 2005; Geffroy *et al.* 2019; Lacey *et al.*, 1991; Poitou *et al.*, 2017), were used as base wines for this study. In both base wines, the best estimate threshold (BET) for olfactive detection of IBMP was also determined to find out the concentration

at which the addition of IBMP can be perceived by panelists.

## MATERIALS AND METHODS

### 1. Base wines and other material

The two commercial unwooded base wines made from pure Sauvignon and Fer (PDO Gaillac) were selected on the basis of absence of any vegetative aroma at tasting. Such wines are typical of those produced in this area during the five last vintages, which are characterised by warm and dry climate conditions.

While performing the olfactory examination, the Sauvignon wine dominantly expressed floral aroma, while the Fer wine was characterised by black fruit and spicy notes. The determination of IBMP concentrations by Nyséos (Montpellier France), with SPME-GC-MS using deuterated IBMP and previously described parameters, (King *et al.*, 2011) revealed negligible levels of this aroma compound: below the quantification limit of 1 ng/L in Sauvignon, and 3 ng/L in Fer. Classical analyses were also performed on the wines using a Winescan FT-120 (Foss France SAS, Nanterre, France), apart from free and total sulfur dioxide, which were determined according to an iodometric method using a Mi455 titrator (Milwaukee Instruments, Rocky Mount, USA). As reflected by the data summarised in Table 1, and notably the level of ethanol, the wines were

**TABLE 1.** Analytical data collected for the two base wines

Parameter	Sauvignon	Fer
Ethanol (% v/v)	14.6	14.9
Titratable acidity (g/L tartaric acid)	5.9	5.5
pH	3.29	3.62
Tartaric acid (g/L)	2.0	1.9
Malic acid (g/L)	0.6	0.1
Volatile acidity (g/L acetic acid)	0.62	0.50
Glucose/fructose (g/L)	0.4	1.7
CO <sub>2</sub> (mg/L)	678	215
DO <sub>420</sub>	0.07	–
MCI <sup>a</sup>	–	12.5
TPI <sup>b</sup>	7.2	75.0
Free SO <sub>2</sub> (mg/L)	22	11
Total SO <sub>2</sub> (mg/L)	72	48
IBMP (ng/L)	<1	3

–Not relevant for this product; <sup>a</sup>Modified colour intensity;

<sup>b</sup>Total phenolic index

produced from fully ripe grapes. This advanced stage of maturity, together with the fact that the two wines were sourced from vintages under warm climate conditions (2018 for Fer, 2019 for Sauvignon), must have contributed to limiting the level of IBMP in finished wines (Roujou de Boubée, *et al.* 2000).

Food grade 3-isobutyl-2-methoxypyrazine (purity > 99 %) provided by Sigma-Aldrich (Saint-Quentin-Fallavier, France) was used to spike the wine for the BET and CRT studies. The spiked wines and the unspiked control wines were prepared 24 h before the tastings. The results of the analyses confirmed that the concentration of added IBMP was appropriate, with a recovery rate of 87–94 %. For all the tasting sessions, samples were coded with three-digit codes, and a constant volume of 15 mL was poured at 18 °C in black and clear wine-tasting glasses for the BET and CRT studies respectively. The tasting sessions took place in a neutral room with white walls and the spacing between the panelists ensured that no communication could occur. The BET and CRT studies were conducted in Toulouse, in the southwest of France, during the last week of February 2020 and the first week of March 2020 respectively.

### 2. Determination of best estimate thresholds

The BET concentrations for olfactive detection of IBMP in Sauvignon and Fer wines were determined according to the American Society of Testing and Materials method E 679 (ASTM, 2004). The panelists (n = 20 for Sauvignon and n = 19 for Fer), were students from École d'Ingénieurs de Purpan aged between 22 and 25 years old, who had previous experience with difference testing and with sensory evaluation of wine. The percentage of female panelists was 65 % and 58 % for Sauvignon and Fer respectively. Due to the different number of male and female panelists, the ASTM E 679 method, which provides individual threshold values in a single session, was preferred to ASTM E 1432 to investigate the impact of gender on the ability to perceive IBMP. The concentrations to be tested were decided following preliminary tasting by a small group of panelists and on the basis of odour thresholds previously reported in the literature (Allen *et al.*, 1991; Roujou de Boubée *et al.*, 2000). The Sauvignon wine, containing

undetectable level of IBMP, was spiked with 1, 2, 4, 8, 16 and 32 ng IBMP/L. The Fer wine, initially containing 3 ng IBMP/L, was spiked with 2, 4, 8, 16, 32 and 64 ng IBMP/L, corresponding to final concentrations of 5, 7, 11, 19, 35, and 67 ng/L. The 3-alternative forced choice (3-AFC) tests, with control wines incorporated, were served in ascending order of IBMP concentrations, as described in the E 679 method. Assessments were only conducted orthonasally (sniffing) without tasting. Panelists had to make a choice, even if no difference was noted. In accordance with the official method (ASTM, 2004), it was assumed that a panelist, who missed at the highest concentration, would have been correct at twice this level. Conversely, it was assumed that a panelist, who succeeded at the lowest concentration, would have been incorrect at half this level. The BET concentrations were first assessed for the Sauvignon wine, and after a 10 min rest for the Fer wine.

### 3. Determination of consumer rejection threshold

The panel was composed of 48 wine consumers recruited during a wine fair that took place at the Ecole d'Ingénieurs de Purpan in Toulouse, in the southwest of France. The participants were not offered any pay for participating in the study and they did not receive any information about the samples they had to evaluate. At the end of the

session, they were asked to provide some demographic information and to respond to several questions regarding their purchase behaviour and their knowledge of vegetative notes in wine. The panel comprised 22 women and 26 men who were between 18 and 24 (31 %), 25 and 34 (23 %), 35 and 44 (4 %), 45 and 54 (23 %) and over 55 (19 %) years old. They reported their habits to be as follows: wine consumption for a mean of 16.4 years (standard deviation = 12.5), every day (6 %), 3 to 4 times a week (40 %), once a week (29 %), twice a month (15 %), and once a month (10 %). They described their level of knowledge as 'no wine knowledge' (23 %), 'interested in wine' (62 %), and 'passionate about wine' (15 %). The questionnaire showed that 56 % of the panelists had previously heard of vegetative aroma in wine, and that 46 % of them appreciated this kind of note.

The procedure for CRT determination was based on a replicate series of five paired comparison tests, one for each IBMP concentration (Prescott *et al.*, 2005). Each pair consisted of a sample of the base wine alone and a sample of the base wine spiked with IBMP. The tasting order was randomised, and each pair was presented in order of ascending concentration. For each base wine, the concentrations were chosen based on the BET study, with the lowest concentration tested being close to the BET value. Panelists were asked to conduct an olfactory assessment, place

**TABLE 2.** Comparison of preference (*P*-value) for each IBMP concentration, for gender, age, level of wine knowledge, familiarity with vegetative notes in wine and self-reported liking of vegetative notes in wine.

	IBMP concentration (ng/L)	Gender <sup>a</sup>	Age <sup>b</sup>	Level of wine knowledge <sup>b</sup>	Familiarity with vegetative notes in wine <sup>a</sup>	Self-reported liking of vegetative notes in wine <sup>b</sup>
Sauvignon	7.5	0.917	0.206	0.051	0.726	0.976
	15	1.00	0.811	0.357	0.251	0.050
	30	1.00	0.417	0.830	1.00	0.497
	60	1.00	0.728	0.227	1.00	0.563
	120	0.950	0.038	0.697	1.00	0.171
Fer	18	0.437	0.811	0.892	1.00	0.110
	33	0.281	0.732	0.694	0.839	0.078
	63	1.00	0.364	0.574	1.00	0.164
	123	1.00	0.956	0.389	1.00	0.750
	243	1.00	0.082	0.564	1.00	0.024

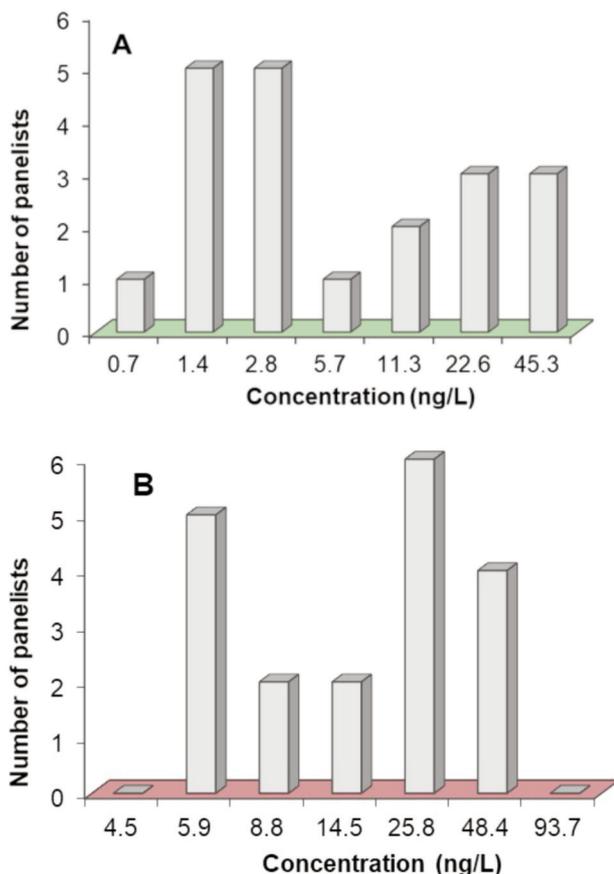
<sup>a</sup>The Kolmogorov-Smirnoff (two-tailed) test was performed; <sup>b</sup>The Kruskal-Wallis test was performed; n = 48 consumers

the whole sample in their mouth, move it around for a few seconds and then spit it out. They then had to indicate the coded sample of the pair they preferred on a form. Panelists received a new pair of samples every five minutes.

#### 4. Data treatment

For each panelist, the BET concentration was calculated as the geometric mean of the concentration at which the last miss occurred and the next higher concentration. The panel threshold was the geometric mean of the BET of the individual panelists, as described in the E 679 method (ASTM, 2004). A Mann-Whitney test was performed to detect any differences in BET according to gender.

The CRT concentration was calculated as the concentration at which a spiked wine was significantly rejected, using the binomial



**FIGURE 1.** Distribution of best estimate threshold (BET) concentration (ng/L) for IBMP in (A) Sauvignon wine (n = 20 panelists) with a global BET at 5.5 ng/L, and (B) Fer wine (n = 19 panelists) with a global BET at 16.8 ng/L, according to the ASTM method E 679

distribution for a paired comparison test (Roessler *et al.*, 1978). The Kolmogorov-Smirnov (two-tailed) test was used to evaluate whether the preference for IBMP differed according to gender and familiarity with vegetative notes in wine. For age, level of wine knowledge and self-reported liking of vegetative notes in wine, the Kruskal-Wallis test was performed. Mann-Whitney, Kruskal-Wallis, and Kolmogorov-Smirnov tests were conducted with XLSTAT software (Addinsoft, Paris, France).

## RESULTS AND DISCUSSION

The panel BET was estimated as being 5.5 ng/L and 16.8 ng/L for Sauvignon and Fer respectively. These results are in accordance with previous studies, emphasising an odour threshold for IBMP of 8 ng/L in Sauvignon (Allen *et al.*, 1991), and 15 ng/L in Cabernet-Sauvignon, Cabernet Franc and Merlot (Roujou de Boubée *et al.*, 2000). The importance of the matrix (i.e., the base wine) on aroma perception in wine has already been highlighted (Robinson *et al.*, 2009; Villamor *et al.*, 2013). These latter reported that increased contents in ethanol, tannin and sugar could reduce the headspace concentration of volatile aroma compounds and threshold values by between 2- and 10,000-fold. As the two studied matrices contained similar levels of ethanol and sugar (Table 1), we can suppose that the larger amount of tannins found in the Fer wine, as reflected by TPI values, is mainly responsible for the BET differences observed between the two wines. The differences in aroma composition between the two wines might also have played a role via mechanisms related to perception interactions (McKay *et al.*, 2020).

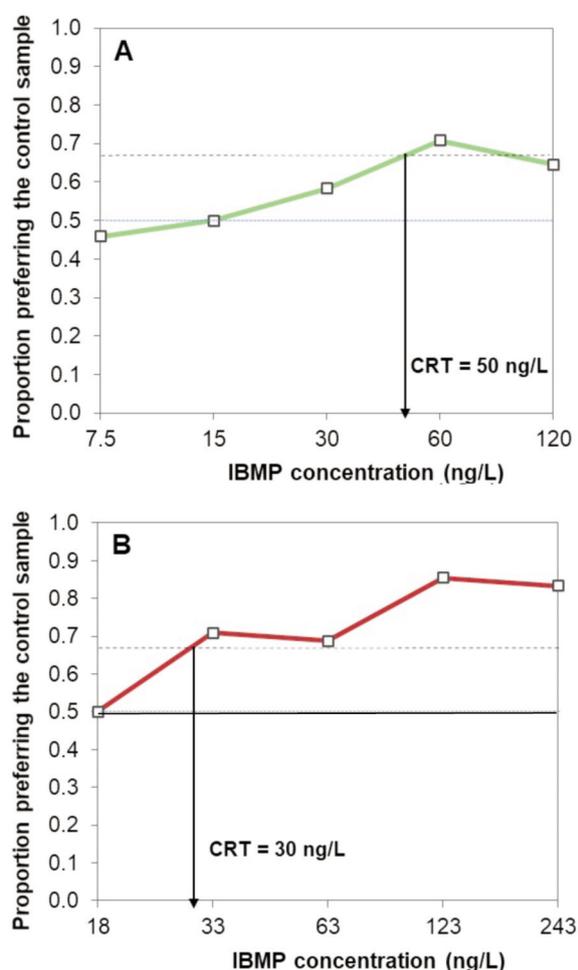
As the panel was only composed of young panelists (< 25 years old), we cannot exclude the fact that slightly higher BETs would have been observed for a more age-balanced population. Indeed, it is well known that sensitivity for detecting odour usually declines with age (Griep *et al.*, 1995). In the same way, as experience is known to facilitate the ability of panelists to discriminate wines when conducting triangular tests (Solomon, 1990), the fact that a less trained panel would have led to a higher BET cannot be rejected.

The distribution of best estimate threshold concentration showed a large variability between panelists and did not follow a normal law

(Figure 1). For Sauvignon, one panelist succeeded at the lowest concentration and three panelists missed at the highest concentration. According to the Mann-Whitney test, no significant differences in BET was observed at  $P < 0.05$  between female and male panelists for Sauvignon ( $P = 0.067$ ) and Fer ( $P = 0.302$ ). However, BETs tended to be lower for female panelists (3.5 ng/L for Sauvignon and 14.6 ng/L for Fer) in comparison to male panelists (21.6 ng/L for Sauvignon and 21.6 ng/L for Fer). Actually, most of the research investigating sex differences in terms of olfactory threshold have reported either greater female sensitivity or no

sex differences in sensitivity, depending on the odorant (Doty and Cameron, 2009). Consequently, the fact that the greater proportion of female panelists within the panel might have contributed to lower the BETs for both wines cannot be completely discarded.

Following these results, the base wines were spiked with IBMP for the CRT study at 7.5, 15, 30, 60, and 120 ng/L for Sauvignon, and at 15, 30, 60, 120, and 240 ng/L for Fer, which, for the latter variety, corresponds to total IBMP concentrations of 18, 33, 63, 123 and 243 ng/L. These concentrations were chosen on the basis that IBMP range should start at levels which are detectable by panelists. For both wines, CRTs were established, at 50 ng/L and 30 ng/L for Sauvignon and Fer respectively (Figure 2).



**FIGURE 2.** Proportion of panelists preferring the control sample in A) Sauvignon wine and B) Fer wine. Horizontal lines show “no preference” (—) and the “control is significantly preferred” at 5 % risk using paired comparison tests (---),  $n = 48$  panelists. CRT stands for Consumer Rejection Threshold according to Prescott *et al.* (2005).

The difference in CRT concentrations between the two matrices indicates that IBMP is more acceptable in Sauvignon wine than in Fer wine. Although the preference was not significant, the Sauvignon wine spiked with 7.5 ng/L of IBMP was preferred to the control (Figure 2A), which is consistent with previous research conducted with Czech panelists (Rajchl *et al.*, 2009). Such IBMP concentration is likely to enhance the freshness of a white wine, a character usually desirable for this kind of product (IFV, 2013). This refreshing effect must have been particularly pronounced as the base wine was made from fully ripe grapes. The results obtained might have been different if other styles of Sauvignon had been used, notably those with high level of varietal thiols, potent aroma compounds that can also contribute to the typicality of wines made from this cultivar (Tominaga *et al.*, 1998). For this latter style of Sauvignon wines, it has been shown that perceptual interactions are likely to reduce the green perception of IBMP (van Wyngaard *et al.*, 2014), which could have led to higher BET and CRT values. However, other studies conducted in Australia on a base wine spiked with high thiol concentrations also demonstrated that the largest cluster of consumers, representing 43 % of the whole panel, preferred Sauvignon blanc wine containing moderate (10 ng/L), rather than high (20 ng/L) levels of methoxypyrazines (King *et al.*, 2011).

It should be noted that the CRT in Sauvignon may not be applicable to all other countries, in particular the countries in which the consumers are familiar with wines containing great

concentrations of IBMP, such as those from New Zealand (Lund *et al.*, 2009). As familiarity is known to positively affect the appreciation of a given aroma by consumers (Saliba *et al.*, 2009), it is possible that such consumers would have rejected spiked samples at higher IBMP concentration.

The lower CRT observed for Fer wine is somehow consistent with research highlighting that French consumers tend to have a short-term preference for Bordeaux red wines with a fully ripe/jammy fruit aroma (for which the sensory contribution of IBMP is likely to be negligible), to the detriment of wines with fresh fruit notes (Tempère *et al.*, 2019). While we targeted red wines made with Fer grapes, our results might be transposable to other red grape cultivars, such as Cabernet-Sauvignon, Cabernet Franc and Merlot, for which the odour threshold of IBMP has been reported at a similar range of concentration (Roujou de Boubée, *et al.* 2000).

For both wines, the proportion of panelists preferring the control wine slightly decreased at between 60 ng/L and 120 ng/L for Sauvignon, and 123 ng/L and 243 ng/L for Fer, which may reflect a saturation of olfactive receptors as a result of the large quantity of aroma compounds inhaled by panelists.

Non-parametric statistical tests on our data showed that the age of panelists had an impact on the appreciation of Sauvignon wines spiked with 120 ng/L of IBMP,  $P = 0.038$  (Table 2). For this IBMP concentration, younger panelists aged between 18 and 24 preferred the control wine in comparison with the other age groups. Although not statistically significant, the same trend was observed for Fer wines spiked with 240 ng/L,  $P = 0.082$ . As our panel had a larger proportion of young panelists, the proportion of panelists which preferred the control might have been overestimated for the highest level of IBMP concentration. For Sauvignon and Fer with 15 ng/L and 243 ng/L of IBMP, the panelists' preference was affected by the self-reported liking of vegetative notes in wines. The wines enriched with IBMP were preferred by the consumers, who stated that they enjoyed the vegetative notes. This coherence between the real appreciation of vegetative notes and consumers' self-reported liking indicates that panelists recruited during a wine fair have good knowledge of wine and are able to analyse their olfactory sensations. It is not clear why this

observation was not confirmed for Sauvignon at 30, 60, and 120 ng/L.

Finally, it should be pointed out that previous IBMP concentrations found in Sauvignon wines have never exceeded the CRT values of 50 ng/L determined in our study. Indeed, a couple of studies have investigated IBMP levels in commercial Sauvignon wines sourced from various countries throughout the world (Lacey *et al.*, 1991; Lund *et al.*, 2009). Outside New Zealand, or other cool climate vineyards such as the Loire valley in France, levels of IBMP rarely exceed 20 ng/L. The highest IBMP concentration of 47 ng/L was recorded in a wine from the Wairarapa region in New Zealand (Lund *et al.*, 2009). As an autochthonous cultivar only grown in the southwest of France, data concerning IBMP in Fer wines are scarce. However, the usual IBMP concentrations also tend to be below the CRT concentration of 30 ng/L, but very high IBMP levels have been previously found in Fer wines; for example, two decades ago in cool climate vineyards (PDO Marcillac) with concentrations of up to 110 ng/L (Davaux, 2005). Indeed, as a result of the expected rise in temperature during the berry development stage, climate change is already decreasing IBMP levels in finished wines (Pons *et al.*, 2017). To our knowledge, in experimental Fer wines made during the last decade without the aim of minimising IBMP content, concentrations did not exceed 27 ng/L, including in those from cool climate vineyards (Geffroy *et al.*, 2015; Geffroy *et al.*, 2019; Poitou *et al.*, 2017). These data from the literature indicate that IBMP is neutrally perceived by consumers in Sauvignon and Fer wines at the usual concentration levels.

## CONCLUSION

In our study, BET and CRT values for 3-isobutyl-2-methoxypyrazine in Sauvignon and Fer wines from Gaillac were estimated. For Sauvignon, these BET and CR thresholds were established as being 5.5 ng/L and 50 ng/L respectively, while for Fer they were 16.8 ng/L and 30 ng/L.

These findings suggest that IBMP is more acceptable in white wine than in red wines. While the IBMP levels reported in the literature for Sauvignon are always below the CRT value estimated for this cultivar, those recorded for Fer during the last decade are also below its

respective CRT value. These observations suggest that IBMP is neutrally perceived by consumers at the usual concentrations in Sauvignon and Fer wines from Gaillac, and this contradicts the belief in the wine industry that consumers find the 'vegetative' styles of wine undesirable, and that IBMP levels should be lowered by any means. Climate change is likely to negatively affect IBMP concentrations in finished wines, with an even more pronounced effect expected over the coming decades (Pons *et al.*, 2017). Therefore, our findings do not necessarily encourage local winegrowers and winemakers to implement production practices to minimise IBMP concentrations in wine and thus reduce the impact of this molecule on the wine sensory profile.

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